



Review

E-cadherin dysfunction in gastric cancer - Cellular consequences, clinical applications and open questions

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ABSTRACT

E-cadherin plays a major role in cell–cell adhesion and inactivating germline mutations in its encoding gene predispose to hereditary diffuse gastric cancer. Evidence indicates that aside from its recognized role in early tumourigenesis, E-cadherin is also pivotal for tumour progression, including invasion and metastization. Herein, we discuss E-cadherin alterations found in a cancer context, associated cellular effects and signalling pathways, and we raise new key questions that will impact in the management of GC patients and families.

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1. Introduction

Gastric cancer (GC) remains a major concern worldwide characterized by an inherent molecular heterogeneity and consequent divergent clinical biology [1]. According to the World Health Organization (WHO) and the Laurén classifications, two main histological types of GC can be described displaying distinct clinicopathological features, the diffuse and intestinal subtypes [1–3]. Diffuse type cancer is composed by non-cohesive cells (with or without signet ring cells) and is more commonly observed in younger patients [3,4]. In contrast, the intestinal type, believed to occur through a multistep progression from multifocal atrophic

gastritis, is characterized by glandular architecture (tubular and/or papillary), and is frequently found in older patients [3,5].

Throughout the last few decades it has become obvious that GC results from a complex interplay between genetics and environment [1,3]. Indeed, it has been well established that GC initiation and progression involves accumulation of genetic alterations in a myriad of genetic pathways, namely those involved in DNA repair, cell adhesion, signal transduction, cell differentiation, and apoptosis, among others [6,7]. Furthermore, different genetic pathways are thought to underlie the development of diffuse- and intestinal-type GCs and given the heterogeneity and complexity of gastric tumours, it is now widely accepted that genetic and epigenetic alterations in the host contribute to the development of disease in combination with environmental factors. Diffuse gastric cancer (DGC) has a clear hereditary form and results from E-cadherin deregulation upon genetic or epigenetic alterations [8], whereas occurrence of intestinal type is more associated with environmental factors such as obesity, dietary factors, cigarette smoking, as well as with infection by *Helicobacter pylori* [1,3,9–11].

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Despite advances in diagnosis and treatment, early stages of disease are often asymptomatic and the late onset of clinical symptoms awards GC patients a poor prognosis [3,12]. Furthermore, curative therapy is only possible upon complete resection of the tumour, usually accomplished with a complete gastrectomy, although subtotal resection of the stomach can be performed depending on the tumour location [13]. In Western countries, it is estimated that 80–90% of GC patients are either diagnosed at an advanced stage when the tumour is no longer operable or develop recurrence within 5 years of surgery [12].

A common and perhaps the most relevant feature of all tumours is the ability of cancer cells to detach from the primary tumour and invade neighbouring and distant sites leading to the formation of metastases and progression of tumour malignancy [14,15]. This capacity arises as cells lose the ability to be adherent and gain an increased potential to invade, a process highly associated to loss of expression of E-cadherin (epithelial cadherin) [16–18]. E-cadherin is a Ca^{2+} -dependent cell–cell adhesion molecule essential for the establishment of epithelial architecture and maintenance of cell polarity and differentiation, both during development and in adult life [19,20]. Extensive research provided evidence that E-cadherin is a broad-acting tumour suppressor, and indeed it is regarded as a major determinant of tumour progression and invasion in epithelial cancers, namely gastric carcinomas [14,20].

In this paper we aim to provide a state of the art report regarding E-cadherin function and dysfunction with specific emphasis on its involvement in GC development and open new avenues in this field of research.

2. E-cadherin structure and function

The prototypical member of the cadherin superfamily was first identified in 1977 by Takeichi as a surface protein with a Ca^{2+} -dependent cell–cell adhesion potential [19,21]. This single-pass transmembrane glycoprotein is encoded by the *CDH1* gene, annotated to the human chromosome 16q22.1 in a cluster along with other cadherins [22]. Transcription is currently annotated at the coordinate 68,771,128 bp and translation (ATG) starts 194 bp downstream [23]. Although the canonical *CDH1* promoter does not contain a TATA box, it displays the highly conserved regulatory elements GC boxes, E boxes and a CCAAT box [19,20].

The mature E-cadherin protein is organized in three major structural domains: a cytoplasmic domain of about 150 amino acid residues (AA), a single transmembrane domain and an extracellular domain of about 550 AA, comprising five tandemly repeated domains exclusive to cadherins, the so-called EC1–EC5 [8,24].

The epithelial cell–cell adhesion is achieved through homophilic interactions between cadherin molecules, first among adjacent cells (*trans*-interaction) and then within the same cell by lateral association (*cis*-interaction), leading to the formation of zipper-like structures [25–27]. Furthermore, E-cadherin conformation is only stable upon Ca^{2+} binding to highly conserved repeats, negatively charged motifs present in the extracellular domain [28,29]. The cytoplasmic domain of E-cadherin interacts with β -, α -, and γ (plakoglobin)-catenins (β ctn, α ctn, γ ctn), with α ctn anchored to the actin cytoskeleton, thus establishing the cadherin-catenin complex [30]. E-cadherin stabilization at the cell membrane and accurate function occurs by association to p120-catenin (p120ctn), which not only accelerates the delivery or recycling of cadherins to the plasma membrane but it also prevents E-cadherin from entering the degrading endocytic trafficking pathway [19,31,32]. The stability of the cadherin-catenin complex, and its linkage to actin filaments, forms the core of the Adherens Junction (AJ), which is vital to inhibit individual epithelial cell motility and to provide homeostatic tissue architecture [8,18].

3. Role of E-cadherin in development

During differentiation processes like gastrulation, neurulation or neurite outgrowth, cell–cell and cell–matrix adhesion must be firm but also flexible enough to allow cell migration and morphogenesis [33]. Indeed, E-cadherin is expressed in all mammalian epithelia and is one of the first adhesion molecules expressed in the mouse embryo. Interestingly, ovumorulin, as it was originally named, was identified at the one cell stage embryo [34,35], and in the preimplantation mouse embryo, at the 8-cell stage, E-cadherin was shown to be essential during morula compaction and subsequent epithelial tissue organization [36]. During fetal development, E-cadherin is only expressed in the embryonic region of the placenta and has been awarded a key role in maintaining a spatial boundary between embryonic and maternal tissues [35,37,38].

E-cadherin knockout (KO) in mice is lethal at embryonic day 4 (E4), resulting in junctional and cytoskeletal organization defects and, ultimately, in failure to form trophoctoderm, the first polarized epithelial layer in the mouse embryo, thus demonstrating its relevance during normal development [39]. Furthermore, germline mutations in *CDH1* have been associated to congenital midline malformations and, in fact, it was proposed that specific E-cadherin alterations during development may underlie the genesis of craniofacial congenital malformations, such as lip and palate cleft [40].

4. E-cadherin regulatory mechanisms

The pivotal role of E-cadherin in specific developmental processes as well as its function during carcinogenesis, discussed below, is reflected in the complexity of mechanisms regulating E-cadherin epithelium-specific expression. Indeed, E-cadherin expression is regulated at many levels, from gene expression to transport and protein turnover at the cell surface [8,41]. During development, many transcriptional repressors have been reported to bind to the E-boxes, inducing downregulation of E-cadherin, namely the zinc-finger proteins of the Snail/Slug superfamily, as well as ZEB1 and ZEB2 [8,42–46]. Another relevant mechanism leading to silencing of E-cadherin is promoter hypermethylation, which has been associated to tumour progression [47–50].

E-cadherin expression is also controlled through positive regulatory elements in the *CDH1* 5' sequence. Examples of activators of E-cadherin are: the hepatocyte nuclear factor-3 (HNF3), the zinc-finger protein WT1 and the transcription factor AP2 [51]. AP2 binds to E-cadherin promoter region, and the retinoblastoma protein (Rb) as well as the proto-oncogene product c-Myc act as co-activators of AP2 in epithelial cells [19].

The exocytic trafficking pathway is fundamental for the correct transport of newly synthesized E-cadherin to the plasma membrane [52]. Once delivered to the cell surface, E-cadherin is regulated by phosphorylation, ubiquitination and proteolysis [53,54].

Integrity of the cadherin/catenin complex is known to be regulated, both positively and negatively, by phosphorylation. Phosphorylation of β ctn by tyrosine kinases, such as Src or other tyrosine kinase receptor (RTK) disrupts the binding to E-cadherin, which is then targeted for degradation [55]. In contrast, serine phosphorylation in the E-cadherin molecule results in an increased affinity to β ctn [56]. O-glycosylation of the E-cadherin cytoplasmic domain has been reported as a mechanism impairing its transport to the cell surface [57], whereas N-glycosylation of E-cadherin is essential for its folding and trafficking [58,59].

Upon its delivery to the membrane, E-cadherin faces another level of regulation, the endocytic pathway, which leads to either its recycling to the plasma membrane, to its transient sequestration inside the cell or to its degradation [54]. Many potential regulators

of E-cadherin trafficking have been identified, namely the ADP-ribosylation factor 6 (Arf6) [60,61] and the E3 ubiquitin ligase Hakai [62]. Interestingly, E-cadherin ubiquitination mediated by Hakai also involves epidermal growth factor receptor (EGFR) activation and Src signalling by Cdc42 [63]. Furthermore, lysosomal targeting of E-cadherin is thought to involve the endosomal sorting activity of HGF-regulated tyrosine kinase substrate (Hrs), as well as Src activation of the GTPases Rab5 and Rab7 [64]. In contrast, p120ctn has been reported as an inhibitor of E-cadherin endocytosis, by blocking adaptor complexes for clathrin-mediated endocytosis [65].

5. E-cadherin dysfunction and gastric cancer

It comes as no surprise that genetic or epigenetic alterations in E-cadherin leads to disturbed epithelial cell-cell adhesion and structure, aberrant stromal interactions, as well as altered cell migration and signalling, with ultimate oncogenic potential [66]. Indeed, functional loss of E-cadherin is a well established molecular event that occurs during tumour progression, leading to increased invasion of cancer cells to neighbouring tissues and to metastasis [67,68]. More so, reduced expression of E-cadherin, mostly due to decreased expression at mRNA and protein levels, is regarded as indicative of poor outcome in a variety of malignancies. In fact, only a minor proportion of advanced carcinomas present *CDH1* mutations [14,69]. *CDH1* is however regarded as a classical tumour suppressor gene in gastric carcinogenesis, being involved in the initiation and progression of both sporadic and hereditary forms of GC [70,71]. Of relevance, inherited germline mutations in *CDH1* are a causative feature of hereditary diffuse gastric cancer (HDGC), awarding E-cadherin as the culprit for the development of this cancer syndrome [72].

HDGC was the first gastric cancer-related disease, identified upon reports of three Maori kindred displaying early onset, multigenerational DGC associated with germline mutations of the E-cadherin gene [73]. Ever since, increasing evidence has further supported a specific role of E-cadherin in the initiation of DGC, and indeed over 40% of HDGC cases present *CDH1* germline mutations [72,74–76]. In 1999, the International Gastric Cancer Linkage Consortium (IGCLC) defined the clinical criteria for identification of HDGC [77], characterized by highly penetrant diffuse-type GC (80% lifetime risk) and elevated risk of lobular breast cancer [72,76]. Familial aggregations with high incidence of GC and with an index case of DGC, although not fulfilling the IGCLC criteria for HDGC, are classified as familial diffuse gastric cancer (FDGC) [4]. Despite that HDGC accounts for only a small subset of GCs (1–3%), it represents a major health issue due to its severity, high penetrance and inefficient surveillance tools [76], and an interesting natural model to unravel E-cadherin-dependent signalling pathways. In addition to the germline *CDH1* mutations observed in inherited DGCs, approximately 50% of sporadic invasive lobular breast carcinomas and diffuse gastric adenocarcinomas display somatic inactivating *CDH1* mutations [78–80].

Germline mutations in *CDH1* have indeed been reported in diffuse early onset gastric cancer (EOGC) patients with and without family history of GC [81]. Corso and colleagues have recently described a summary of apparently sporadic diffuse or mixed cases revealing that, in fact, 7.2% in fact carried *CDH1* germline mutations, of which 2.3% were predicted to be deleterious [81].

Remarkably, Masciari et al. reported *CDH1* germline mutations associated with invasive lobular breast cancer in the absence of DGC, despite that it is widely accepted that *CDH1* germline mutations are infrequent in women with early-onset or familial lobular breast cancers independently of DGC association [82,83].

6. Molecular deregulation of E-cadherin

To date, 122 germline mutations have been described in the *CDH1* gene [84], and although there are no major mutational hot-spots, some mutations (1003C > T, 1901C > T and 1137G > A) have been consistently identified in unrelated kindred [72]. In a recent metanalysis by Corso et al., a significant association was found between *CDH1* mutation frequency and low-risk areas for GC. Specifically, from a set of 122 *CDH1* germline mutations, 87.5% arose from low-risk areas, although the majority of which were of the non-missense-type, whereas in high-risk areas, missense mutations were predominant (11/16; 68.8%). This interesting finding suggests that ethnicity of GC patients should be considered a significant risk factor for gastric carcinoma and confirms that GC indeed presents various clinic-pathologic and molecular features [84]. Strikingly, germline *CDH1* mutations are rarely found in countries such as Japan and Korea [72], although this effect can be related to the high rates of sporadic GC found in these countries. The most common types of *CDH1* germline mutations are small frameshift insertions and deletions, as well as point mutations, with 80% resulting in protein truncation or even complete loss of expression. The remaining 20% of *CDH1* germline alterations are of the missense type [85–89], whose pathogenic relevance and functional assessment implicates a myriad of *in silico* and *in vitro* functional assays developed by our group [90–93]. Additionally, we reported for the first time in 2009, germline *CDH1* large genomic deletions in apparently mutation negative HDGC families [74]. Somatic, E-cadherin mutations are mainly splice site mutations resulting in exon skipping (frequently exons 8 or 9) [78,94].

Development of DGC in patients harbouring *CDH1* mutations (hereditary and sporadic) occurs upon a “2nd-hit” mechanism that leads to E-cadherin aberrant or absent expression [95–97]. Hypermethylation in a large CpG island in the *CDH1* 5′ proximal promoter is an important epigenetic event associated with loss of E-cadherin gene expression and occurs in a myriad of carcinomas [66,98]. In HDGC tumours, hypermethylation of the *CDH1* promoter is considered the most common mechanism associated to biallelic *CDH1* inactivation, accounting for 50%–70% of the cases. Moreover, our group has reported that loss of heterozygosity (LOH) represents a key mechanism in metastatic lesions from HDGC patients [49,95]. In sporadic forms of diffuse gastric carcinomas, promoter methylation is also regarded as the most frequent “2nd-hit” inactivating mechanism [80].

Despite the widespread belief that classical *CDH1* inactivation mechanisms are predominantly associated to diffuse-type of GCs, abnormal E-cadherin expression has been reported in the majority of GCs of both diffuse and intestinal types [99]. MicroRNAs (miR) have emerged as a potential mechanism regulating *CDH1* expression thus contributing to gastric carcinogenesis [100], and for instance, members of the miR-200 family have been associated to regulation of E-cadherin expression, by targeting the transcriptional repressors ZEB1 and ZEB2 [101,102]. Strikingly, our group has recently identified miR-101 down-regulation as a new mechanism underlying E-cadherin inactivation mainly in intestinal-type tumours [103].

Aside from genetic and epigenetic silencing of E-cadherin, many other mechanisms can account for E-cadherin dysfunction during pathological conditions. Aberrant regulation of E-cadherin trafficking pathways lead to disruption of its function and, consequently, to pathophysiological conditions, such as malignant transformation and cancer metastases [54]. Accordingly, we recently demonstrated the relevance of E-cadherin trafficking deregulation in two E-cadherin missense mutations associated to HDGC [104]. We showed that mutant E-cadherin is targeted for proteasomal

degradation upon recognition by the Endoplasmic Reticulum Associated Degradation (ERAD) machinery [104].

E-cadherin dysfunction during tumour development and progression has also been associated to *N*-glycosylation and *O*-glycosylation modifications [58,105]. Particularly, we have reported aberrant *N*-glycosylation modifications associated to E-cadherin deregulation in human breast and GCs [106]. On the other hand, abnormal activation of proto-oncogenes, including c-Met and Src, has also been shown to result in increased phosphorylation of tyrosine residues in the cytoplasmic domain of E-cadherin, leading to the recruitment of Hakai and subsequent ubiquitin-degradation of E-cadherin [62].

7. E-cadherin mediated tumour spectrum

CDH1 germline mutations confer more than 80% lifetime risk to specifically develop GC, albeit that E-cadherin is ubiquitously expressed in all epithelial tissues. However, the tissue specific molecular determinants behind the predominant increased risk for stomach cancer remain to be uncovered. Our group has previously reported that GC cells silenced for E-cadherin are more resistant to pro-apoptotic stimulus than control cells, in a Notch signalling-dependent manner [107,108]. Moreover, extensive analysis of normal tissues expression arrays further revealed that 40% of normal gastric-specific genes are involved in survival/apoptosis, most of which have been reported to be deregulated in GC, such as the Trefoil Factor Family 1 (TFF1) [109]. Thus, we hypothesize that in the stomach, cells negative for E-cadherin are able to overcome apoptosis and ultimately become more prone for transformation due to a specific genetic programme or to microenvironmental settings. Our preliminary data indicates that cumulative activation of Notch and gastric-specific genes may be underlying the ability of gastric

cells to overcome E-cadherin mediated apoptosis in contrast to other tissues (unpublished).

8. E-cadherin related signalling pathways

Throughout the past decades many molecules and signalling pathways have been associated to E-cadherin regulation and function. Increasing evidence suggests that a network of signalling pathways intersects with E-cadherin and these are known to involve a multitude of molecules including EGFR, Notch-1, Bcl-2, Rho family members and matrix metalloproteinases (MMPs) as illustrated in Fig. 1. Aberrant E-cadherin will therefore promote deregulation of E-cadherin-mediated signalling pathways, having an impact in cell-cell adhesion, migration, invasion and survival [8].

As an adhesion molecule, E-cadherin interacts with a wide range of cellular partners as catenins, integrins, growth factor receptors and cytoskeletal components to mediate intracellular signalling and modulate the organization of the cytoskeleton, crucial for the maintenance of cell polarity [110]. Of particular interest is the convergence of the β catn and cadherin pathways. In addition to its central role as an adaptor protein linking cadherins to the actin cytoskeleton in cell-cell adhesion, β catn is also a key player in the Wnt signaling, acting as a transcription cofactor with T cell factor/lymphoid enhancer factor (TCF/LEF) [111]. However, the role of E-cadherin in modulating the Wnt/ β catn remains to be dissected. Results from our group have shown that E-cadherin mutants affecting the β catn binding domain, and resulting in E-cadherin loss of function, do not lead to nuclear accumulation of β catn or increased cell proliferation, suggesting that the effect of the E-cadherin mutants does not induce the activation of the Wnt pathway in a β catn-dependent way [112].

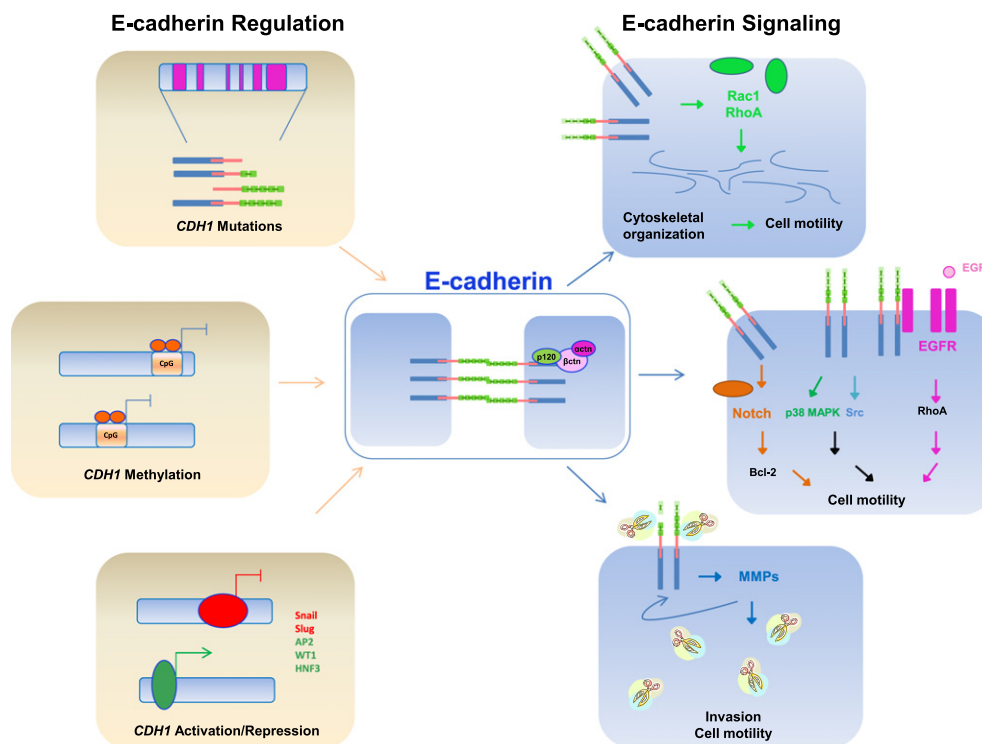


Fig. 1. Schematic overview of proposed mechanisms of *CDH1* regulation and E-cadherin mediated signalling pathways involved in gastric cancer. E-cadherin expression is deregulated at various levels in gastric cancer. *CDH1* inactivating mutations, promoter methylation and transcriptional regulation, through activators and repressors, are frequently found in gastric cancer. Aberrant E-cadherin expression will in turn promote deregulation of E-cadherin-mediated signalling pathways having an impact in cell-cell adhesion, migration, invasion and survival. The network of signalling pathways that intersects with E-cadherin involves, among others, Rac1, RhoA, EGFR, Notch, Bcl-2, and MMPs.

Another signalling pathway frequently deregulated in GC involves Rho GTPases, known to play a role in cytoskeletal organization. For instance, Rac1 and RhoA were shown to be overexpressed in primary gastric carcinoma [113]. Furthermore, increased RhoA activity, which led to higher migration capacity, was induced by HDGC-associated extracellular E-cadherin missense mutations [90,114]. Moreover, EGFR has been shown to be involved in RhoA activation in an E-cadherin-dependent manner [114,115]. Noticeably, we demonstrated that mutations at the E-cadherin extracellular domain impair the EGFR/E-cadherin interaction, leading to EGFR activation, and enhanced cell motility through activation of RhoA [114].

As for EGFR, other pathways relevant for cell motility such as Src kinase and p38 MAPK have been shown to be aberrantly activated as a consequence of HDGC-related E-cadherin mutations [116]. In addition, alterations in the expression of MMPs, important to modulate the surrounding extracellular matrix (ECM) and facilitate tumour invasion and metastasis, have also been described in GC. For instance, reduced expression of E-cadherin and increased expression of MMP1 and MMP2 was found in GC tissues, for which E-cadherin loss and MMP2 expression was strongly correlated with deeper tumor invasion [117]. Likewise, a negative correlation between MMP9 and E-cadherin was found in a set of gastric carcinomas [118]. A functional link between MMP3 and E-cadherin has also been found in transfected cells showing that MMP3 is differentially regulated by expression of the wild type or mutant E-cadherin variants, with subsequent effects on cell motility [119]. In addition, using *in vitro* models, our group observed a regulation of MMP activity by E-cadherin. Mutant E-cadherin cells secreted higher levels of MMP9, in contrast to cells expressing the wild type protein (unpublished data). Of particular interest is the fact that E-cadherin can be suppressed by MMPs, through cleavage in the E-cadherin extracellular domain, thus adding an extra level of control in the invasion process [120].

E-cadherin has also been shown to be involved in apoptosis and cell survival. Indeed, we have shown that functional loss of E-cadherin renders cells more resistant to apoptotic stimuli [107]. Strikingly, using *in vitro* and *in vivo* studies, we have demonstrated that E-cadherin impairment is able to increase cell survival through Notch-dependent upregulation of Bcl-2 [108].

9. Pathogenesis of *Helicobacter pylori*

H. pylori infection is widely regarded as a major etiologic factor for the development of gastric carcinoma [121]. Mounting evidence has associated this bacterial pathogen with the epigenetic silencing of E-cadherin [122,123] and with other mechanisms responsible for its deregulation, namely cleavage of E-cadherin extracellular domain by ADAM10 or by the secreted bacterial protease HtrA [124,125] and, although controversial, translocation of E-cadherin/catenin complex proteins from the membrane to intracellular locations [126–128]. Interestingly, our group has demonstrated that, in E-cadherin wild type cells, *H. pylori* infection promotes the formation of a multiproteic complex containing c-Met and E-cadherin, impairing *H. pylori* induced c-Met-mediated signaling [129]. However, in E-cadherin defective GC cells *H. pylori* infection induces c-Met activation and increases the activity of MMP2 and MMP9, thus leading to ECM degradation and subsequent cell invasion, which may contribute to the increased susceptibility for *H. pylori*-infected individuals to develop GC [130].

10. *In vivo* cancer models

Thus far, the most compelling *in vivo* evidence supporting the causal relationship between E-cadherin loss of function and initia-

tion of DGC was reported by Humar et al. in 2009. For the first time, the authors successfully induced signet-ring cell carcinomas (SRCCs) in *CDH1*^{+/-} mice using a known stomach carcinogen (N-methyl-N-nitrosourea) [75]. Other reports include a conditional E-cadherin KO targeted to stomach parietal cells. Although clusters of signet ring-like cells were found, no invasive gastric adenocarcinomas were observed in E-cadherin-deficient mice, reinforcing the idea that other interactors may be involved in E-cadherin-mediated DGC development [131]. Just recently, Shimada and co-workers established the first genetically engineered mouse model of DGC through conditional KO mice for both E-cadherin and p53. The animals displayed intramucosal and invasive cancers composed of poorly differentiated carcinoma and signet-ring cells, and furthermore, their gene expression profiles resembled those of human patients [132].

Other *in vivo* studies aimed to unravel how E-cadherin's loss can lead to metastases, namely ovary metastases as described in HDGC patients. In mice, human GC cell lines displaying abnormal E-cadherin expression lead to ovary metastases, and the rescue of E-cadherin expression completely inhibited the ovarian-metastatic phenotype, without interfering with the metastatic ability to other organs [133].

The study of HDGC-associated E-cadherin missense mutations has also been successfully undertaken using fruitflies. Expression of these mutated proteins in the *Drosophila* wing epithelium mimicked the *in vitro* results, both in terms of migration and invasion [134]. A *Drosophila* approach to screen for novel GC-related genes was recently reported by our group in a background sensitized by E-cadherin mutations [135].

Conversely, rescue of E-cadherin expression in cancer cell lines and in transgenic mice models of carcinogenesis impaired invasion and reversed poorly differentiated carcinoma phenotypes to a well-differentiated epithelioid phenotype, providing mounting evidence for the tumour suppressor role of E-cadherin [136–138].

11. Pro-oncogenic role of soluble E-cadherin

The identification of E-cadherin as tumour suppressor is undoubtedly associated to the predisposition to both diffuse gastric and lobular breast cancers in patients harbouring mutations in the *CDH1* gene [139]. Furthermore, the widespread notion is that epithelial-mesenchymal transition (EMT) induction and loss of E-cadherin is a prerequisite for metastasis and disease progression. However, it has been suggested that E-cadherin may play alternative roles in tumour progression, namely through stabilization of cell contacts during “collective cell migration” [140], as well as aberrant cytoplasmic and nuclear signalling [18]. Proteolytic cleavage and release of soluble E-cadherin fragments is proposed to exert a pro-oncogenic effect enhancing tumour growth, survival and motility [18,68]. The clinical detection of elevated soluble E-cadherin fragment (sE-cad) in the sera of cancer patients with poor prognosis may award sE-cad a prognostic value for invasive and/or metastatic disease. Nonetheless, this remains a controversial subject and in fact, a study by Pedrazzani et al. suggests that sE-cad should not be recommended in the diagnosis and management of GC patients, since they observed a correlation between E-cadherin serum levels and age [141].

In contrast to normal cells where E-cadherin degradation is regulated by endocytosis, in cells undergoing apoptosis, proteolysis of E-cadherin occurs by MMPs, namely ADAM10 and ADAM15, which have been reported to be upregulated in a variety of cancers awarding E-cadherin cleavage fragments a potential “tumourigenic” role [68,142]. Nonetheless, systematic and rigorous research is still lacking to describe the specific mechanisms underlying the putative role of E-cadherin in tumour promotion.

12. E-cadherin germline mutations and clinical implications

According to the recommendations of the IGCLC, prophylactic gastrectomy should be offered to asymptomatic carriers of *CDH1* truncating germline mutations, as the role of endoscopic surveillance in clinical management of the disease remains controversial. Indeed, the identification of small foci of signet-ring cell gastric carcinoma (early DGCs) in prophylactic gastrectomy specimens, from asymptomatic mutation carriers not yet displaying endoscopic evidence of disease, raises concerns regarding the efficacy of the current surveillance protocols [72,91,143]. Despite its limitations, endoscopic surveillance is still recommended for mutation carriers younger than 20 years old or for those who decline or delay prophylactic surgery, and should be carried out annually [72].

In hereditary forms of GC, carriers of *CDH1* germline missense mutations represent a major burden in terms of genetic counselling and clinical management, and thus, there have been increasing efforts to predict the pathogenic significance of *CDH1* germline missense variants. Noticeably, a multidisciplinary approach involving the study of mutation co-segregation with disease within pedigrees, frequency in healthy population controls, recurrence in unrelated families, as well as *in silico* and functional *in vitro* assays is currently used to identify high-risk individuals for HDGC [74,85,91,92]. Moreover, very recently, we have reported a complementary method using proximity ligation assays (PLA) to assess the interplay between E-cadherin missense mutants and regulators of E-cadherin trafficking, thus unveiling the pathogenicity of E-cadherin missense mutations [93]. Currently, we are developing bioimaging tools to determine the profile of E-cadherin expression at the cellular level. This analysis will extract morphological and textural features from immunofluorescence images and will allow the characterization of wild type and mutant E-cadherin expression.

13. Therapeutic approaches to E-cadherin mediated tumours

As discussed above, aberrant or absent E-cadherin expression in the majority of GCs is consistent with bi-allelic dysfunction of the *CDH1* gene. Accordingly, the identification of putative targets to restore E-cadherin expression has been a continuous challenge. DNA demethylating agents in combination with other epigenetic drugs (such as HDAC inhibitors) are potentially attractive to control the development of disease given that they would prevent new DNA methylation events as well as inhibit other epigenetic events which may precede promoter hypermethylation, such as histone modification [144,145]. This type of approach expected to restore *CDH1* expression seems applicable only in small foci of the primary tumour displaying epigenetic alterations. In contrast, genetic alterations usually acquired as the tumour progresses could be targeted with drugs such as EGFR and Notch inhibitors which have been shown to suppress cell apoptosis, migration and metastasis [108,114,146].

14. Concluding Remarks

E-cadherin has been awarded a key role during metazoan development, where coordinated cell-cell adhesion is required for proper establishment of the body plan and integrity of tissue differentiation. Mounting evidence has arisen over the last decades establishing E-cadherin as a tumour suppressor, and in fact, suppression of E-cadherin function and/or expression has been widely associated to an EMT phenotype and increased cell migration and invasion [66,147]. Strikingly, E-cadherin germline mutations have been identified in a large subset of HDGC kindred and are nowadays regarded as the cause underlying DGC development [73,76].

The recognition of E-cadherin as a key molecule at the intersection of cell-cell adhesion, cell morphology and polarity, and cell life and death, has awarded E-cadherin a leading position in cancer initiation and progression. Understanding and identifying the underlying molecular mechanisms of cadherin-dependent signalling will undoubtedly have an impact in the clinical management of cancer patients, namely GC patients, and in the identification of most effective molecular targets for treatment.

Future research should focus in the quest for other tumour suppressor genes and oncogenes expected to play a cumulative role, along with E-cadherin, in the pathophysiology of GC. The development of new bioimaging tools to survey carriers of E-cadherin germline mutations should also be on the spotlight, as well as effective therapeutic regimens to treat patients with carcinomas mediated by E-cadherin alterations at an early stage of disease progression.

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